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DESCRIPTION AND IMPLICATIONS FOR ANALYSIS OF BRAIN GROWTH IN SUCKLING MICE

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The growth pattern of the brains of normal CD-1 mice was studied from day 1 through day 21 after birth. The main purpose of the study was to decide on suitable methods of statistical analysis of these kinds of data including whether to analyze brain weight or the brain-to-body weight ratio. A secondary purpose, which aided the main purpose, was to find functions that describe the relationships between growth parameters. Linear-linear segmented polynomials were found to describe well the relationships between (a) brain weight and body weight, (b) brain weight and age, (c) brain-to-body weight ratio and body weight and (d) brain-to-body weight ratio and age. The analyses indicated that brain weight alone is the preferred variable to analyze. Regression analysis of brain weight with sex and up to the first three powers of body weight and age as independent variables would be a suitable method of analysis. Litter size per se had no effect on brain weight.

INDEX TERMS: Brain growth; brain growth analysis; Mouse CD-1.

INTRODUCTION

One frequent measurement made to assess the toxic effects of agents in developing animals is organ weights (Benitz, 1970; Weil, 1969; Wilson, 1973). The proportional size of various organs can be instrumental in uncovering mechanisms of growth retardation in fetuses. For example, brain retardation may be accompanied by similar reductions in other organs when prenatal undernourishment is the cause, but the brain is not effected as other organs are when the prenatal agent is vascular insufficiency of the uterus (Winick, 1976).

The fetal and neonatal brain appear to have lower growth rates when hyperthermic agents raise the maternal temperature during gestation. Edwards and Wanner (1977) conclude that the fetal central nervous system is extremely vulnerable to hyperthermia induced in maternal guinea pigs by higher ambient air temperatures. Fetal rats are reported to have smaller brains after gestational exposure to another hyperthermic agent, radiofrequency radiation (Shore *et al.*, 1977). Lately, it has been shown that radiofrequency radiation exposure during gestation can cause a decrement, possibly permanent, in the postnatal growth of the brain in mice (Berman *et al.*, 1985).

However, it is not clear what measurement should be used to examine the growth of an organ such as the brain. Because organ size is expected to vary in proportion to body size, brain weight, for example, is often expressed as the brain weight divided by the body

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weight (Shore *et al.*, 1977; Berman *et al.*, 1985). Forming this brain-to-body weight ratio theoretically eliminates that part of the variability in brain weights that can be attributed to differences in body weights. It is also suggested that the brain-to-body weight ratio be further adjusted by using body weight as a covariate (Edwards and Wanner, 1977). Further, it appears that litter size and litter weight may also influence brain weight (Zamenhof and van Marthens, 1984).

The literature does not give a picture of a clear-cut pattern of brain growth in mice; the only available data are those of Kobayashi (1963). He used Wistar mice, a different strain than we had employed (Berman *et al.*, 1985), and concluded that an abrupt change in the brain-to-body weight ratio occurs at approximately 14 days. So that we might pursue our studies of the effects of radio frequency radiation on brain growth in CD-1 mice, we investigated the normal growth pattern of the brain of this strain of mouse from day 1 through day 21 after birth, and the influence that age, litter size, sex, and body weight have on brain weight. Functional forms for the relationships of brain weight and other variables are suggested. The implications of these findings for the analysis of brain weight are discussed.

Materials and Methods

Time-bred, primiparous CD-1 mice (Charles River Laboratories, Kingston, NY) were allowed to deliver. At either 1, 5, 10, 13, 15, 17, 19 or 21 days following delivery, the entire litter was sacrificed. Each individual was sexed, weighed, killed by decapitation, and the brain was then removed and weighed. Only litters where all individuals survived to sacrifice were used. By extending the neck during decapitation and angling the cut of the blade perpendicular to the spinal cord at this point, the brain, when removed, included the nervous tissue just posterior to the cerebellum. The weighed sample included the olfactory bulbs, but not the pituitary.

Results

There were 17 to 22 litters, with 182 to 232 individuals, examined at each of the eight days (Table 1). The sample sizes at each day were similar both for litters and individuals. The mean and standard error of body weight, brain weight and brain-to-body weight ratio based on individuals and the mean and standard error of litter size are given in Table 2 for each day. Mean litter size was similar for each day. The population, therefore, appears to be similar at each experimental point. Sample sizes, means and standard errors for brain weight, body weight and their ratio for each sex are in Table 3. Litter incidences and percent females for litter sizes 5 through 15 (all the litters in this study) are, respectively: 2, 30.0%; 2, 50.0%; 7, 51.0%; 13, 55.8%; 26, 49.1%; 25, 50.0%; 29, 49.8%; 26,

Table 1. Number of CD-1 mice litters and individuals at each age.

Age when killed (Days)	Number of Litters	Individuals
1	17	182
5	19	202
10	22	232
13	20	213
15	19	201
17	20	209
19	20	204
21	20	214
Total	157	1657

46.8%; 14, 54.9%; 10, 53.6%; 3, 46.7%. The overall mean litter size was 10.6 pups; 50.3 percent were females.

The relationships between brain weight and body weight and between the brain-to-body weight ratio and body weight are shown by the plotted points in Figures 1 and 2, respectively, where each point represents one mouse. Inspection of the plotted points shows that the relationships are not linear. Brain weight increases relative to body weight in an apparently linear relationship up to a body

Table 2. Mean (SE) body weight, brain weight, brain-to-body weight ratio for individuals and litter size for each age. Sample sizes are the same as in Table 1.

Age when killed (Days)	Body Weight (g)	Brain Weight (g)	Brain/Body Ratio	Litter Size
1	1.62	.097	.0599	10.7
	(.012)	(.0005)	(.0004)	(.54)
5	3.07	.183	.0601	10.7
	(.030)	(.0012)	(.0004)	(.48)
10	5.77	.334	.0587	10.5
	(.060)	(.0020)	(.0004)	(.48)
13	7.09	.399	.0568	10.6
	(.054)	(.0011)	(.0004)	(.49)
15	7.16	.411	.0586	10.6
	(.082)	(.0016)	(.0006)	(.53)
17	8.46	.429	.0517	10.5
	(.098)	(.0015)	(.0005)	(.52)
19	10.05	.442	.0446	10.2
	(.093)	(.0014)	(.0004)	(.45)
21	12.38	.456	.0374	10.7
	(.119)	(.0016)	(.0003)	(.40)

Table 3. Sample size and mean (SE) body weight, brain weight and brain-to-body weight ratio for each age and sex.

Age Killed (Days)	FEMALES N	Body Weight (g)	Brain Weight (g)	Ratio	MALES N	Body Weight (g)	Brain Weight (g)	Ratio
1*	82	1.61 (.018)	.097 (.0007)	.0604 (.0005)	99	1.64 (.014)	.097 (.0006)	.0592 (.0004)
5	112	2.99 (.039)	.180 (.0016)	.0607 (.0005)	90	3.17 (.044)	.186 (.0019)	.0592 (.0005)
10	122	5.81 (.083)	.334 (.0029)	.0583 (.0006)	110	5.74 (.088)	.334 (.0029)	.0592 (.0006)
13	95	6.93 (.070)	.396 (.0017)	.0576 (.0005)	118	7.23 (.079)	.401 (.0015)	.0562 (.0005)
15	104	6.95 (.116)	.405 (.0024)	.0598 (.0009)	97	7.40 (.111)	.416 (.0021)	.0573 (.0007)
17	97	8.49 (.156)	.426 (.0022)	.0515 (.0007)	112	8.44 (.124)	.430 (.0021)	.0520 (.0006)
19	113	9.96 (.126)	.440 (.0018)	.0449 (.0005)	91	10.17 (.136)	.445 (.0023)	.0443 (.0005)
21	108	12.27 (.177)	.453 (.0024)	.0376 (.0004)	106	12.49 (.160)	.459 (.0021)	.0372 (.0004)

*Sex unknown for one mouse

weight of about 6-7 g. After this point, brain weight appears to increase in a linear relationship with body weight, but at a slower rate. The brain-to-body weight ratio appears to be fairly constant up to a body weight of about 4-6 g; thereafter, it decreases in a slightly non-linear fashion as body weight increases.

The plotted points in Figures 3 and 4 show the relationships between brain weight and time from birth (in days) and between the brain-to-body weight ratio and time, respectively. Brain weight appears to increase linearly with time up to about 12-13 days; thereafter, it appears to increase linearly with time, but slower. The brain-to-body weight ratio appears to have a slight linear decrease as time increases up to about 14-16 days; thereafter, it appears to decrease linearly, but more rapidly.

Discussion

The data in the four figures suggest that a segmented polynomial (Gallant and Fuller, 1973) could be used to describe the relationship between each pair of variables. The plotted points in each figure appear to have two segments with each segment being linear with the possible exception of the second segment in the brain-to-body weight ratio and body weight graph (Figure 2). The two segments meet at the join point which is the estimate of the point at which the

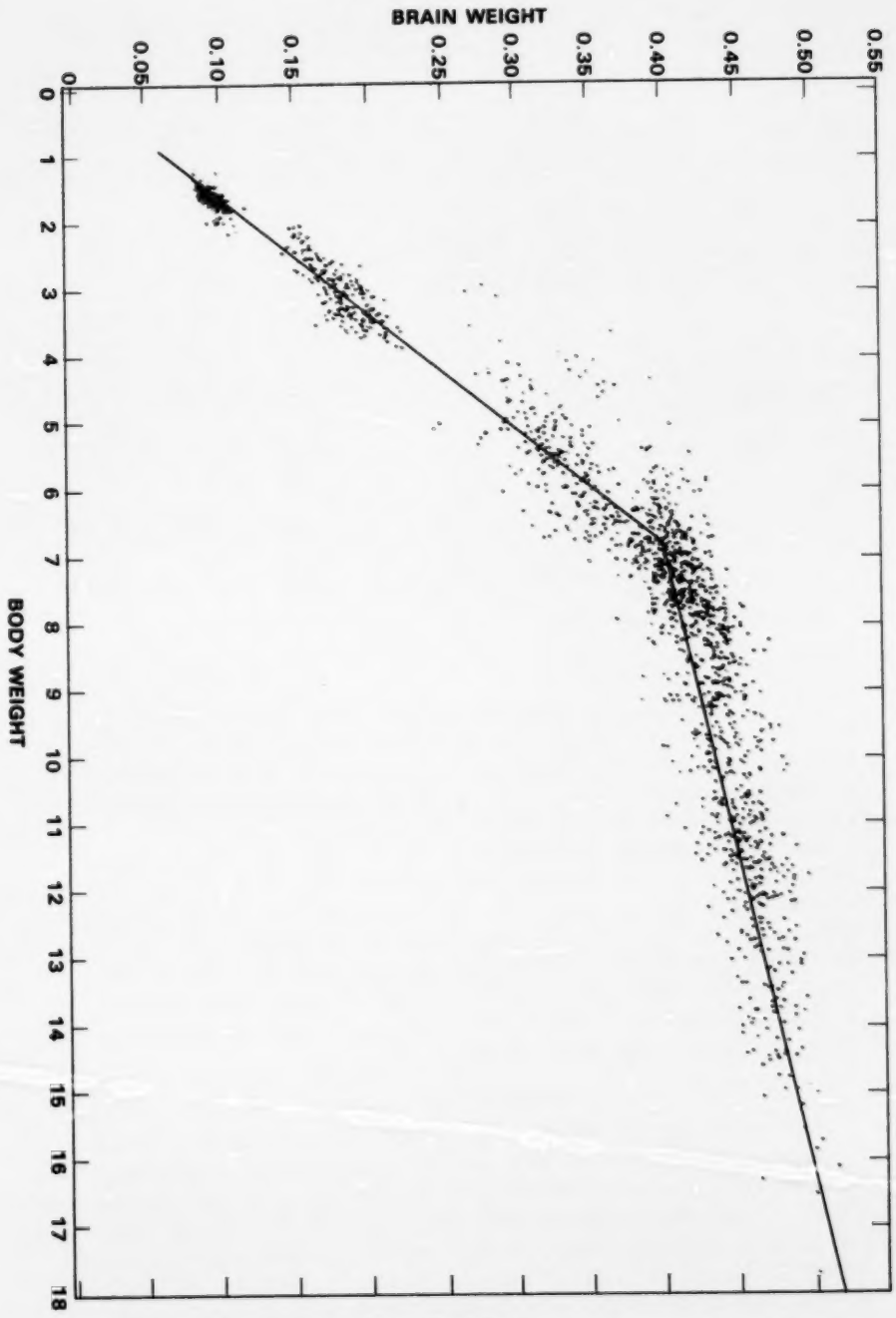

FIGURE 1

Relationship between brain weight and body weight. Each point represents one mouse. Line is best fitting linear-linear segmented polynomial. $R^2 = .969$. Equation of line is: Brain weight = .0044 + .0597 (Body weight); Body weight < 6.67. Brain weight = .3345 + .0102 (Body weight); Body weight > 6.67. 95% confidence interval for join point (JP) is: $6.56 < JP < 6.78$.

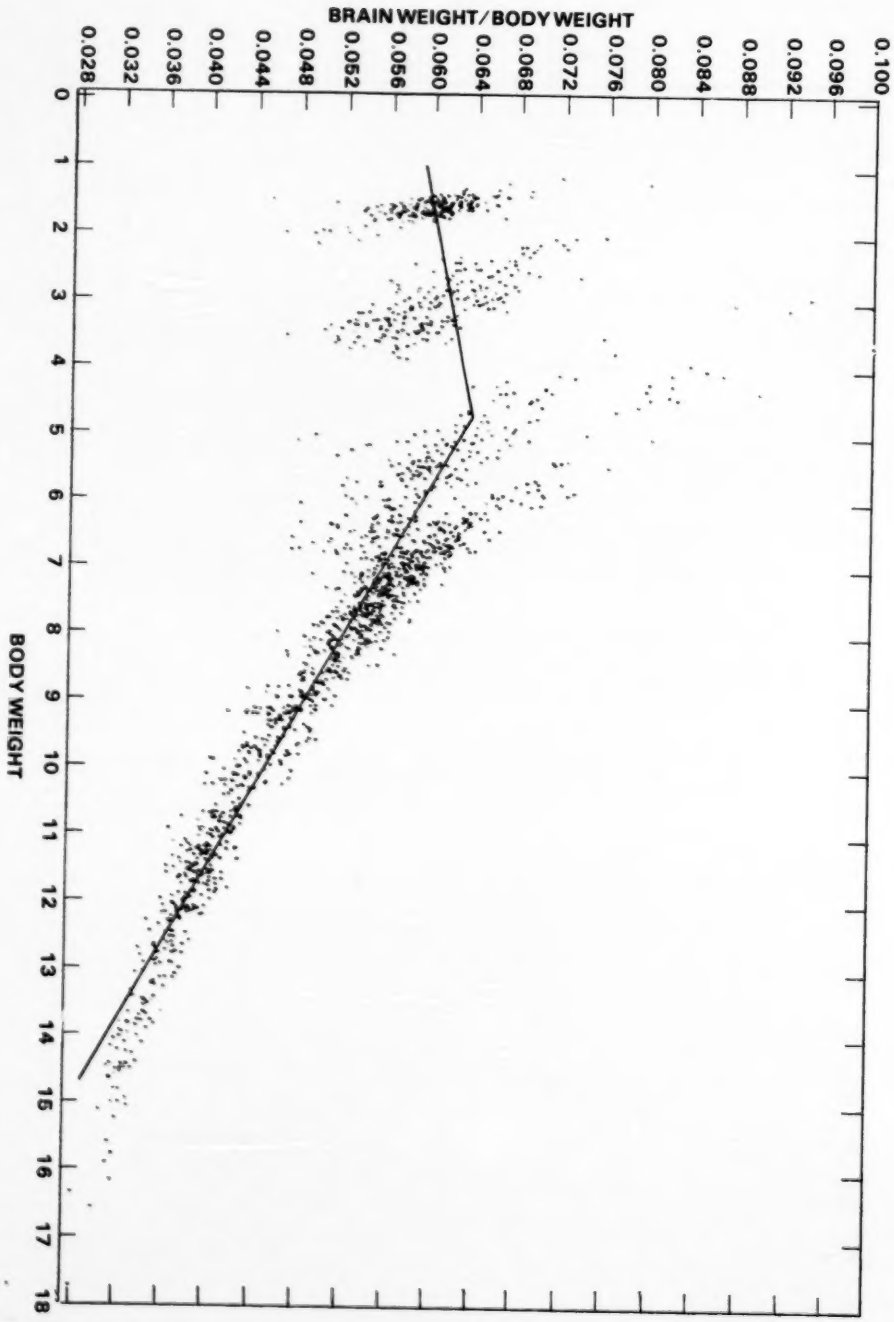

FIGURE 2

Relationship between brain-to-body weight ratio and body weight. Each point represents one mouse. Line is best fitting linear-linear segmented. polynomial. $R^2 = .799$. Equation of line is: Ratio = .0581 + .00129 (Body weight); Body weight < 4.71. Ratio = .0808 − .00351 (Body weight); Body weight > 4.71. 95% confidence interval for join point (JP) is: 4.52 < JP < 4.95.

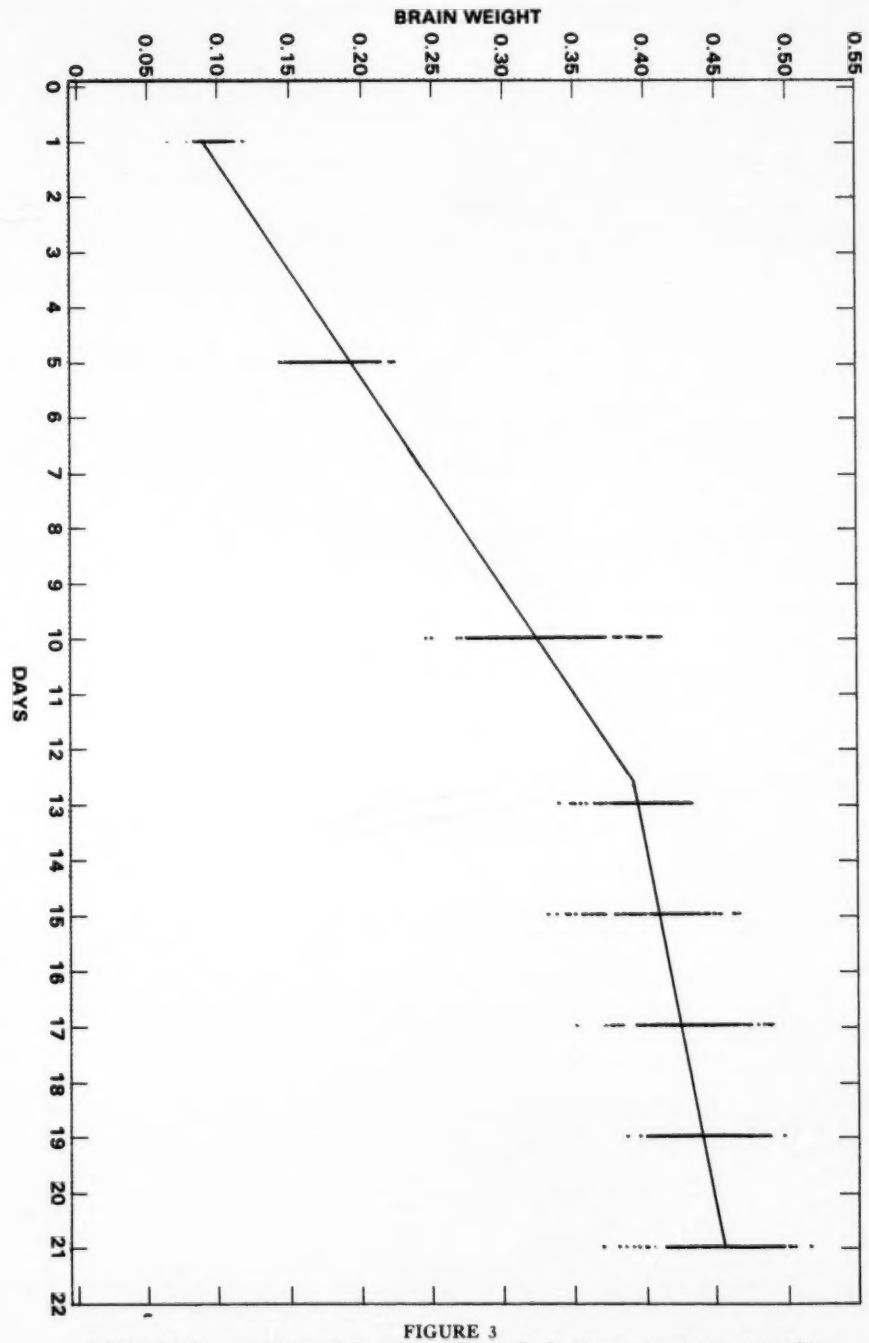

FIGURE 3

Relationship between brain weight and time (Days). Each point represents one mouse. Line is best fitting linear-linear segmented polynomial. R^2 = .967. Equation of line is: Brain weight = .0621 + .0267 (Days); Days < 12.44. Brain weight = .3037 + .00727 (Days); Days > 12.44. 95% confidence interval for join point (JP) is: 12.21 < JP < 12.67.

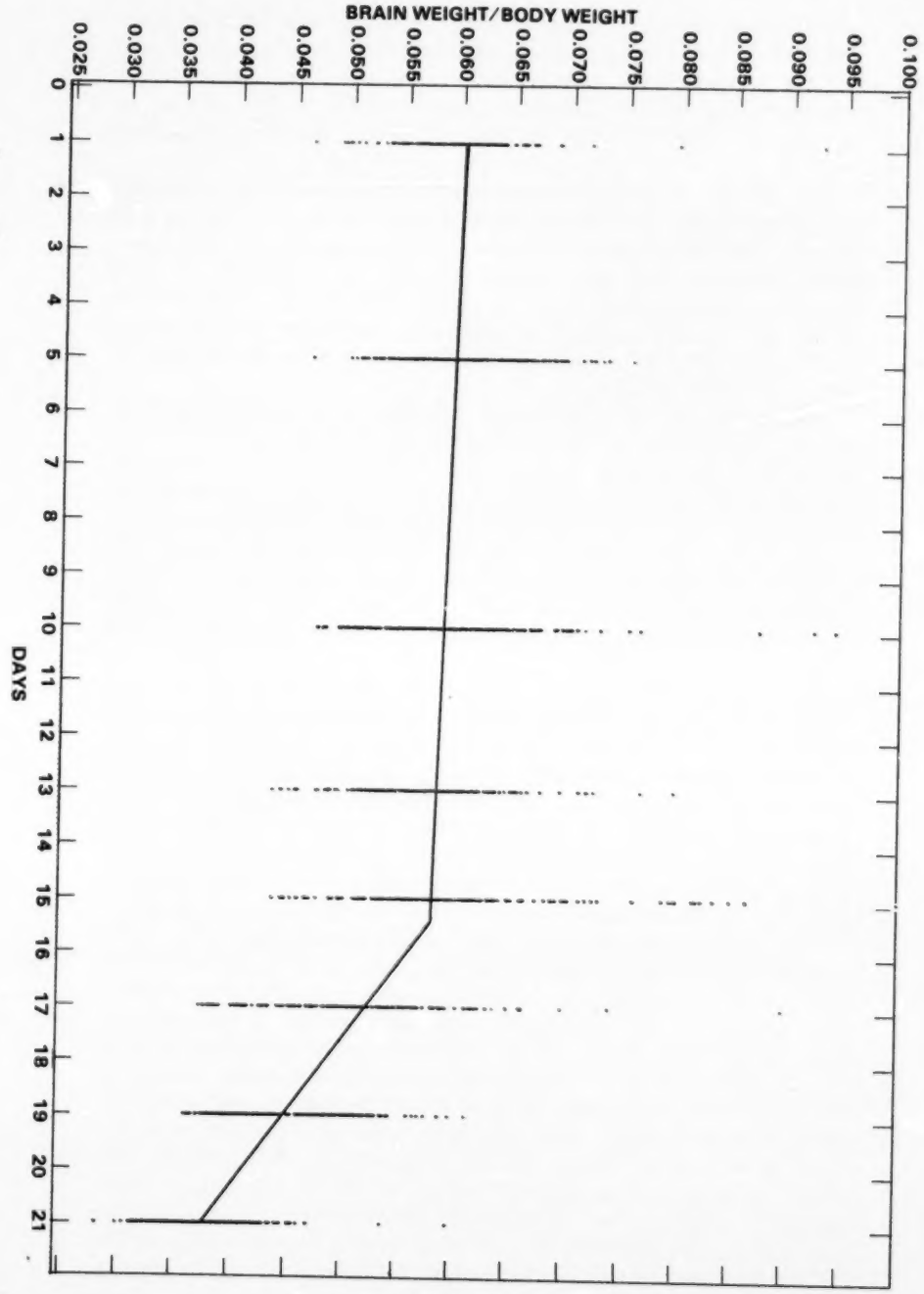

FIGURE 4

Relationship between the brain-to-body weight ratio and time (Days). Each point represents one mouse. Line is best fitting linear-linear segmented polynomial. $R^2 = .627$. Equation of line is: Ratio = .0604 − .00018 (Days); Days < 15.36. Ratio = .1127 − .00359 (Days); Days > 15.36. 95% confidence interval for join point (JP) is: $14.98 < JP < 15.72$.

relationship changes. A segmented polynomial with both segments linear (denoted as a linear-linear segmented polynomial) was fitted to the data in each figure and the resulting equations are given with the corresponding figure. The line in each figure is the plot of the polynomial. The estimate of the join point, its' 95% confidence interval and R^2 are also given with each figure.

The R^2s of the fitted segmented polynomials are 0.969 and 0.967 for brain weight vs. body weight and brain weight vs. time, respectively. The linear-linear segmented polynomial appears to fit these data extremely well since about 97% of the variability in brain weights can be accounted for by either body weights alone or time alone. For the brain-to-body weight ratio, the corresponding R^2s are 0.799 and 0.627. Obviously, these R^2s are not nearly as good as those for brain weight.

The change in the relationship between brain weight and body weight is estimated to occur when the mouse body reaches 6.67 g. For the brain-to-body weight ratio, the relationship with body weight is estimated to change at 4.71 g. A linear-quadratic segmented polynomial for this latter relationship would have a join point nearer 6.67 g and would fit the data slightly better. The relationship between brain weight and time is estimated to change during the 13th (12.44) day after birth and for the brain-to-body weight ratio, the change is estimated to occur during the 16th (15.36) day.

For each of the four pairs of variables discussed above, a linear-linear segmented polynomial was fitted to the data for each sex. In each case, the difference in the polynomial for males and the one for females appeared to be small. The log-log model was also fitted to each of the four pairs and in every case the resulting R^2 was less than that obtained for the linear-linear segmented polynomial. Therefore, it seems that the linear-linear segmented polynomial calculated on the combined data for both sexes is a reasonable model to describe the relationship between each of the four pairs of variables.

The above analyses show the strong relationships that brain weight has with body weight and time and the strong relationships that the brain-to-body weight ratio has with each of these variables. This means that for any analysis of brain weight or the brain-to-body weight ratio, body weight and time must be controlled in order to make the analysis as statistically powerful as possible. Designing an experiment with predetermined levels of body weight would be difficult or impossible. However, regression analysis does afford a method of analysis where this is not necessary. Body weight and time would be used as independent (or predictor) variables in these analyses. Further, since none of the relationships is linear, adding additional independent variables such as the second and possibly higher powers of age and body weight would be necessary to remove

as much variation as possible in either one of the two possible dependent variables (brain weight or the brain-to-body weight ratio).

A regression analysis using all the data in this study was done with sex and the first three powers of age, body weight and litter size as predictors of brain weight and the brain-to-body weight ratio. The results are given in Table 4. The table gives for each predictor the estimate of the coefficient and the significance level of that estimate. Litter size is not a significant predictor of either brain weight or the brain-to-body weight ratio since none of the first three powers was significant. The intercept is significant for the brain-to-body weight ratio ($p < .001$), but not for brain weight ($p = .532$). Brain weights are about .002 g heavier for males than for females, a small but statistically significant difference ($p = .010$). The brain-to-body weight ratio was not significantly different between the sexes ($p = .233$). The remaining predictors are significant ($p < .05$) for both brain weight and the brain-to-body ratio variables with the possible exception of the first power of age as a predictor of brain weight which had a p-value of 0.052. The R^2 for brain weight is 0.985 and for the brain-to-body weight ratio it is 0.920. This indicates that brain weight might be better than the brain-to-body weight ratio as the dependent variable. If more variability can be accounted for using brain weight, then for a given sample size a more statistically powerful experiment is possible. Or, for the same power, an experiment analyzing brain weight would need a smaller sample size than one analyzing the brain-to-body weight ratio. This finding is in agreement with the analyses done for our earlier report (Berman *et al.*, 1985) where in every case the p-value for the exposure group effect was less when brain weight was analyzed than it was when the brain-to-body weight ratio was analyzed.

The powers of body weight and age that would be significant predictors would depend on the range of values of those variables that are used in a particular experiment. For example, if only the first 10 days of brain growth are being investigated, using powers of age other than the first would probably not be necessary since the relationship of brain weight and age is linear through the first 12 days. Likewise, if only body weights over 7 g were expected, then only the first power of body weight would probably be necessary since the relationship of brain weight and body weight is linear after about 6.7 g. Powers of body weight or age other than the first will probably be necessary if the range of values of that variable includes the join point. If the join point is near the center of expected values of body weight or age, then as the range increases higher powers of that variable should be considered.

In Figures 1 and 2, there are noticeable clusters of points at the lower values of body weight. Figures 5 and 6 provide insight into the nature of these clusters by showing the linear component of the

Table 4. Results of regression analysis.

	Brain Weight		Brain-to-body Weight Ratio	
	Coefficient Estimate	P	Coefficient Estimate	P
Intercept	.0172	.532	.0725	<.001
Body Weight	.0583	<.001	-.0139	<.001
(Body Weight)^2	-.00402	<.001	.000746	<.001
(Body Weight)^3	.000112	<.001	.0000137	<.001
Age	.00219	.052	.00491	<.001
Age^2	.0014	<.001	.0000475	.002
Age^3	.0000579	<.001	.00000403	<.001
(Litter Size)	-.00842	.304	-.000158	.918
(Litter Size)^2	.00111	.165	.0000619	.676
(Litter Size)^3	.0000394	.118	.00000252	.592
Sex	.0019	.010	.000165	.233
R^2	.985		.920	

relationship between each pair of variables for each day of age that measurements were made. Each line is plotted over the range of body weights observed for that age. It is obvious from these figures that the clusters correspond to the days measurements were made. In both figures, the clusters for the first three days of measurement

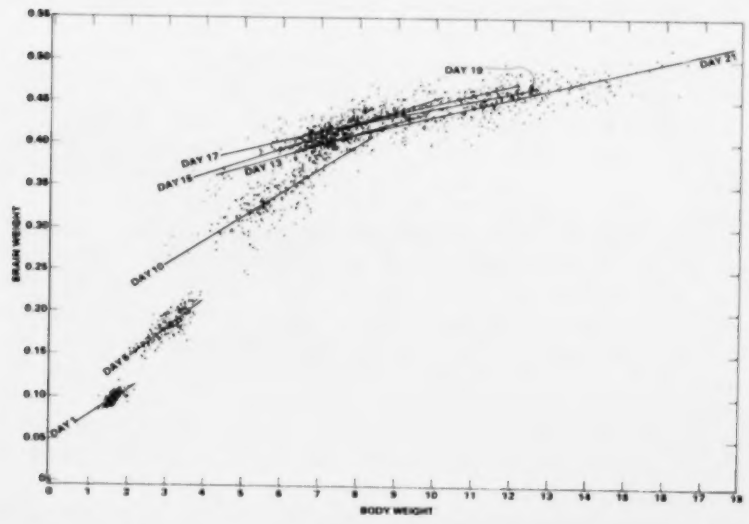

FIGURE 5
Relationship between brain weight and body weight for each day animals were killed. Each point represents one mouse. Best fitting straight line is shown for each day.

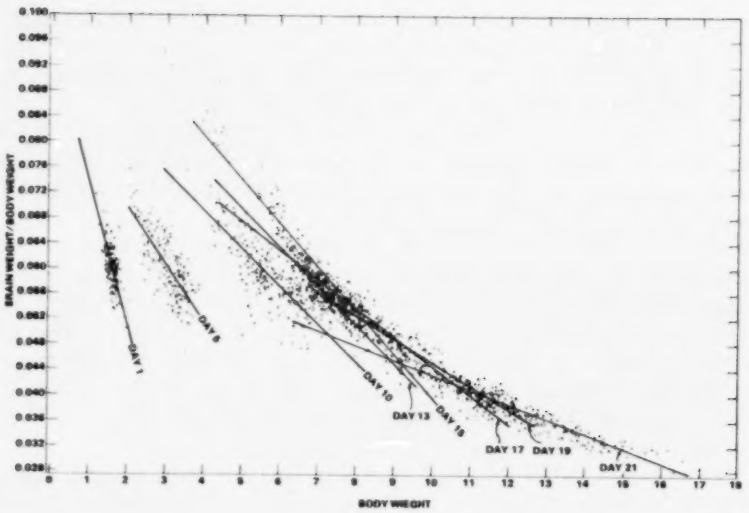

FIGURE 6
Relationship between brain-to-body weight ratio and body weight for each day animals were killed. Each point represents one mouse. Best fitting straight line is shown for each day.

are distinct while those for the last five days of measurement are more overlapping. It is important to note that the lines for brain weight vs. body weight at individual days can be reasonably well deduced from the estimated segmented polynomial in Figure 1. This is, however, not the case for the brain-to-body weight ratio, at least for the early days (or small body weights), where the first segment of the segmented polynomial has a small positive slope (body weights <4.71 g) while for the first few days that have body weights in this range, the slopes are all comparatively large and negative. The relationship between the brain-to-body weight ratio and body weight over the whole range investigated in this study does not give a true indication of the relationship of those two variables during the early days. However, for brain weight alone, the relationship as estimated by the segmented polynomial does indicate fairly well the relationship for the individual days.

Kobayashi (1963) has described the growth of the mouse brain. He used an outbred strain of mice (Swiss Wistar), while we used a different outbred strain (CD-1). His data were based only on litters that were reduced to six pups at birth. To further reduce variation in body weight of offspring, he used only those whose body weights fell within a range obtained from three other litters. In any case, Kobayashi's data indicate that the relationship between the brain-to-body weight ratio and time changes at about 14-15 days. From our data and analyses, we estimate 15.4 days for this same join

point, a value that differs possibly because of our more exact methods or because of strain differences. It is our conclusion that Kobayashi's technique of restricting litter size did not account for any differences between the two estimates.

In our analyses, we have shown that brain weight is the preferred dependent variable mainly because in every situation where the same analysis was done on both brain weight and the brain-to-body weight ratio, the R^2 from the analysis of brain weight was larger than the R^2 from the analysis of the brain-to-body weight ratio. This conclusion is also supported by the analyses done for our earlier report (Berman *et al.*, 1985), which was mentioned previously. We have also shown that litter size per se has little influence on brain weight (Table 4). Sex, body weight and age do, however, have significant influences on brain weight. Therefore, our suggestion for variables that need measurement would include brain weight, body weight, age and sex.

The lack of influence that litter size has on brain weight can be important when designing experiments. For example, the practice of normalizing litter size, whether by culling down to a specific average number, or by fostering extra pups from large litters to small litters, or by using litters of only one natural size, can be avoided if the body weight of the pup is measured. This represents a great economy as all litter sizes can be used and left with their natural mothers.

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